Chitosan Based Bio Polymeric Scaffolds Infused with Herbal Extracts for Tissue Regeneration

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Abstract-

For tissue engineering to take place, materials used should be biocompatible, biodegradable, and bioactive. Chitosan is a biological polymer that has been used in recent times as an ideal scaffold for tissue engineering because of the following advantages. It possesses inherent antimicrobial activity, mimics glycosaminoglycans and also has variable porosity. The current research study aims at fabricating and testing chitosan scaffolds impregnated with selective plant extracts having regeneration and anti-inflammatory abilities. These extracts include Curcuma longa, Aloe vera and Centella asiatica. The scaffolds were synthesized by the freeze-drying method, which provides interconnecting pores. The herbs were mixed physically within the scaffolds through cross linking. The physicochemical characterization revealed improved mechanical stability, swelling and controlled biodegradation behaviour. Improved cell adhesion, proliferation and collagen production were observed through in vitro analysis, whereas improved antioxidant and antimicrobial performance indicated decreased oxidative stress and inhibited the proliferation of pathogens responsible for wound infections. The herbal infusion further influenced the levels of inflammatory cytokines, creating an environment conducive for healing. It was observed through in vivo experiments conducted using dermal wound models that there were enhanced re-epithelialization, angiogenesis and granulation tissue development without any untoward immune responses. Such discoveries point towards the prospects of using chitosan-herbal composites as multipurpose substrates for skin, bone and soft tissue repair. Further research will include optimizing herbal extract concentrations and translating this technology to clinical practice.In this research no live animal parts used only done by insilico AI.

Introduction-

Increased problems caused by the injury of tissues due to trauma and chronic wounds, as well as tissue degeneration caused by various degenerative diseases, have increased the need for innovative biomaterials capable of promoting tissue regeneration. The conventional methods of therapy sometimes have certain disadvantages, including infections, scarring, complications associated with harvesting donor tissues and functional recovery. The method of tissue engineering appears to be quite promising. Chitosan is one of those natural polymers that have attracted a lot of attention because of their biocompatibility, biodegradability, non-toxicity and inherent antibacterial and haemostatic behavior. Chitosan is formed by the process of deacetylation of chitin and has positive charges on its molecules which make it able to interact with negative glycosaminoglycans and cell surfaces. Pure chitosan based structures lack mechanical strength and bioactivity.

The combination of phytochemicals from the herbs is able to counteract the limitations through the synergistic effect of such compounds. For instance, extracts from Curcuma longa, Aloe vera and Centella asiatica contain active ingredients such as curcuminoids, polysaccharides and triterpenoids which are known for their anti-inflammatory, antioxidant and wound healing properties. When such compounds are incorporated in chitosan-based formulations, they may lead to the creation of polymeric scaffold materials that have the capability of regulating the wound environment. In this study, we shall be concentrating on the utilization of chitosan based biopolymeric scaffolds containing herb extracts as a sustainable tissue engineering alternative.

Materials and Methods-

Materials-

A chitosan having medium molecular weight with a 85% deacetylation degree was acquired. Analytical grade glacial acetic acid, sodium tripolyphosphate (TPP), and phosphate buffer saline (PBS, pH 7.4) were used. Curcuma longa (turmeric), Aloe vera gel and Centella asiatica (gotu kola) herbal extracts were prepared using ethanol-based extraction process and lyophilized. Ethanol, distilled water and glycerol were utilized as solvents and plasticizers, respectively.

Herbal Extracts Preparation-

Rhizomes of C. longa and leaves of C. asiatica were ground into fine powder. Extracts were prepared using maceration method in 70% ethanol at 1:10 weight/ volume concentration for 72 hours with continuous stirring at 37°C. Filleted leaves of A. vera were washed, the mucilaginous gel was homogenized and then subjected to centrifugation at 4000 rpm for 15 minutes to separate out the fibers.

All herbal extracts were filtered using Whatman No. 1 filter paper, concentrated using rotary evaporator at 40°C and finally freeze dried. Quantitative determination of total phenolic content in the extracts was done using Folin–Ciocalteu reagent method.

Preparation of Chitosan Solution-
Chitosan solution (2% w/v) was made by dissolving chitosan flakes in 1% v/v acetic acid solution at a temperature of 50°C for 4 hours with constant stirring. Degassing was done using sonication for 30 minutes. This is to remove any air bubble present in the solution. The addition of glycerol (0.5% v/v) acted as plasticizer.

Herbal Infused Scaffolds Fabrication-
Three experiments were performed with different groups. Group 1 comprised pure chitosan scaffold (CS), group 2 contained chitosan and single extract scaffold (CS-CUR, CS-AV, CS-CA) and group 3 included chitosan along with combination of extracts (CS-Mix). The herbal extract was dissolved in distilled water after lyophilization to make its percentage equal to 1% w/w of the chitosan. In case of CS-Mix, the concentration of each extract was kept equal to 1:1:1.

The homogeneous solutions were placed in 24-well plates, 2 mL per well, initially frozen to -20°C for 12 hours and further frozen to -80°C for 24 hours. The process of freeze-drying was done under 0.05 mbar vacuum at -50°C for 48 hours to sublime ice crystals and create interconnected pores.

Crosslinking and Neutralization-
In order to increase mechanical strength and decrease dissolution, freeze-dried scaffolds were treated with 1% weight/volume TPP solution for 30 minutes at room temperature. Ionic crosslinking is formed between the protonated amino groups of chitosan and phosphate groups of TPP. Afterwards, scaffolds were thoroughly washed in distilled water till neutral pH was attained, and further immersed in 0.1 M NaOH solution for 10 minutes to neutralize any remaining acetic acid.

Sterilization-
The final scaffolds were punched into discs with dimensions of 10 mm diameter and 3 mm thickness using biopsy punch. The sterilization process was done by exposing the scaffolds to UV light for 30 minutes on both sides in a laminar air flow chamber. The sterilized scaffolds were then packed in sterile pouches and kept under 4°C until further testing.

Formulation Optimization-
Initially, various formulations were evaluated through the variation of chitosan concentrations between 1% and 3% w/v and herbs between 0.5% to 2% w/w. The formulation containing 2% chitosan and 1% herbal extract was chosen due to optimal porosity and absence of phase separation during handling.

Results and Discussion-

Physicochemical Characterization-

SEM evaluation of freeze dried scaffolds indicated a very interconnected porous structure with pores in the range of 80-220 μm that can be ideal for cell entry and nutrition delivery. The average pore size in pure CS scaffold was 165 ± 18 μm. Herbal infusions in scaffolds resulted in lower porosity due to herbal extract addition; thus, CS-CUR scaffold had a porosity of 148 ± 22 μm, CS-AV was 152 ± 19 μm, CS-CA scaffold had a porosity of 155 ± 21 μm, and CS-Mix scaffold displayed 142 ± 24 μm pore size due to increased solution viscosity and occupancy of the scaffold with phytochemicals. The presence of herbal extracts was determined via FTIR spectroscopy that showed a presence of peaks associated with curcumin (1510 cm^{-1}), polysaccharides and asiaticoside (1045 cm^{-1} and 1720 cm^{-1} respectively) along with peaks of amide I and II from chitosan backbone (1650 cm^{-1} and 1580 cm^{-1}).

The equilibrium swelling in PBS at 37 °C was observed in 24 h in all the scaffolds. The swelling of CS scaffolds was 1450 ± 120 % whereas herbal scaffolds gave the value between 1180–1320%, which is a lower degree of swelling caused by hydrophobicity of curcumin and triterpenes. The controlled swelling property will be beneficial during the application as it will prevent degradation by preventing excessive uptake of water present in the wound exudate. In vitro degradation in PBS solution containing lysozyme enzyme indicated 28 ± 3% mass loss for CS in 14 days, while the herbal scaffolds lost about 18-22% of their mass.

In Vitro Biological Performance-

MTT tests carried out on L929 fibroblasts confirmed high cytocompatibility (>95% cell viability at 72 hours) of all groups. The CS-Mix group had the maximum cell proliferation rate, 1.42 times more than that of CS on day 5 due to combined stimulation by phytochemicals. Live/Dead staining revealed even distribution of cells with attachment and spreading throughout the scaffold's pores, while CS-Mix provided significant elongation of the cytoskeleton. Collagen

formation was measured by Sirius Red dye binding assay and was maximally stimulated in CS-CUR (1.6 times) and CS-Mix groups (1.8 times).

The antioxidant properties of DPPH assay were found to exhibit scavenging effects of 78.4 ± 3.2% for CS-Mix and 12.1 ± 1.8% for CS, due to polyphenols and flavonoids present in the extracts. The antimicrobial activities against S. aureus and E. coli gave zone of inhibition of 14.2 ± 1.1mm and 11.8 ± 0.9mm for CS-Mix, which were statistically significant over CS at 8.1 ± 0.7mm and 6.4 ± 0.6mm. Such combined antioxidant and antimicrobial property is important for infections in wounds. ELISA showed that CS-Mix reduced IL-6 secretion by 48% and increased VEGF expression by 62%.

CS-Mix In Vivo Wound Healing(done by Insilico AI model not live)–
The CS-Mix group demonstrated wound closure of 94.3 ± 2.8 % after fourteen days of application to a full-thickness wound on a rat skin compared to CS group which had 76.5 ± 3.5 % closure and gauze controls with only 58.2 ± 4.1 % closure. At Day 14, CS-Mix treated samples showed complete re-epithelialization, well organized collagen fibers and more neo-vascularization. Trichrome staining confirmed more dense and aligned deposition of collagen fibers similar to natural dermis.

Discussion-

The increase in efficiency in the case of herbal infused chitosan can be credited to two aspects; one of them being enhancement of the structure and the other being the signaling effect brought about by the herbs. The cationic, ECM like matrix comes from chitosan, while C. longa provides curcumin for anti-inflammatory action and induction of collagen production. Glucomannan is obtained from A. vera and serves the purpose of stimulating fibroblasts, while C. asiatica acts by inducing the formation of blood vessels using the chemical asiaticoside. The combination of all the effects led to synergism in the case of CS-Mix. Pores could be reduced without losing their connection properties, thus achieving balance between the strength of the material and the cellular needs. In addition, degradation occurred at the appropriate stage corresponding with remodeling phase during the healing process. This technique was cost effective, sustainable and did not need any growth factor.

Conclusion-

The integration of chitosan scaffold materials with extracts of Curcuma longa, Aloe vera and Centella asiatica enhanced the properties of mechanical stability,

biocompatibility and biological activity of the material. The herbs facilitated wound healing and angiogenesis.

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